NORTHWESTERN
UNIVERSITY

Multi-mode X-ray Studies

Stuart R. Stock

Institute for Bioengineering and
Nanoscience in Advanced Medicine

Echinoderms 101:
Sea urchins, sand dollars, starfish

~ or ~

Preparation for

your vacation at the beach

~ or~

Remediation for those of you

who already did the beach thing

Sorry if you are spending your holiday here in the Midwest, there are no echinoderms here, outside of aquaria.

You might go canoeing or sailing on the lake here…

So something completely different is offered as a brief digression.

Approach

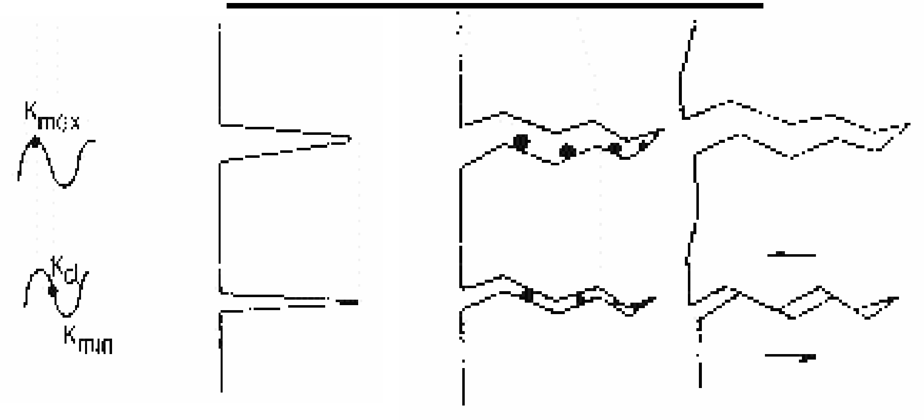
- Repeated observation of the same structure as it alters.
- Extraction and interpretation of 3D structure.

- Absorption microCT (tube-, synchrotron-based)
- Microbeam x-ray diffraction mapping, < 0.05 mm dia.
- X-ray phase imaging (synchrotron x-rays)

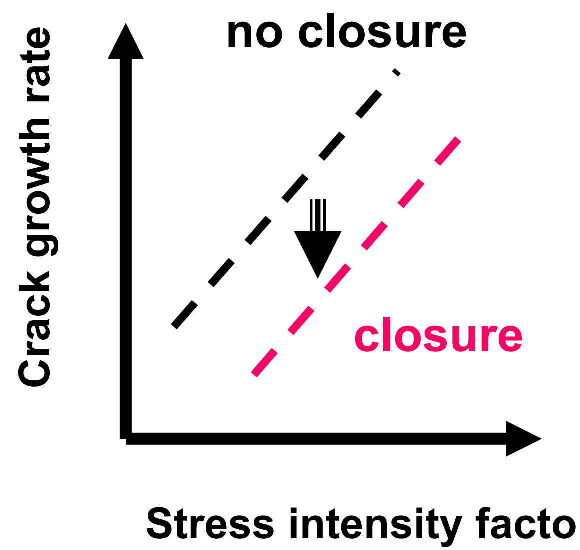
A Trip to the Dark(?) Side

- Fatigue crack closure in engineering alloys (Al)
- Premature contact during unloading (prolonged contact during loading) thought responsible for up to 10X decrease in fatigue crack growth rate !!!

<u>Closure mechanisms</u>



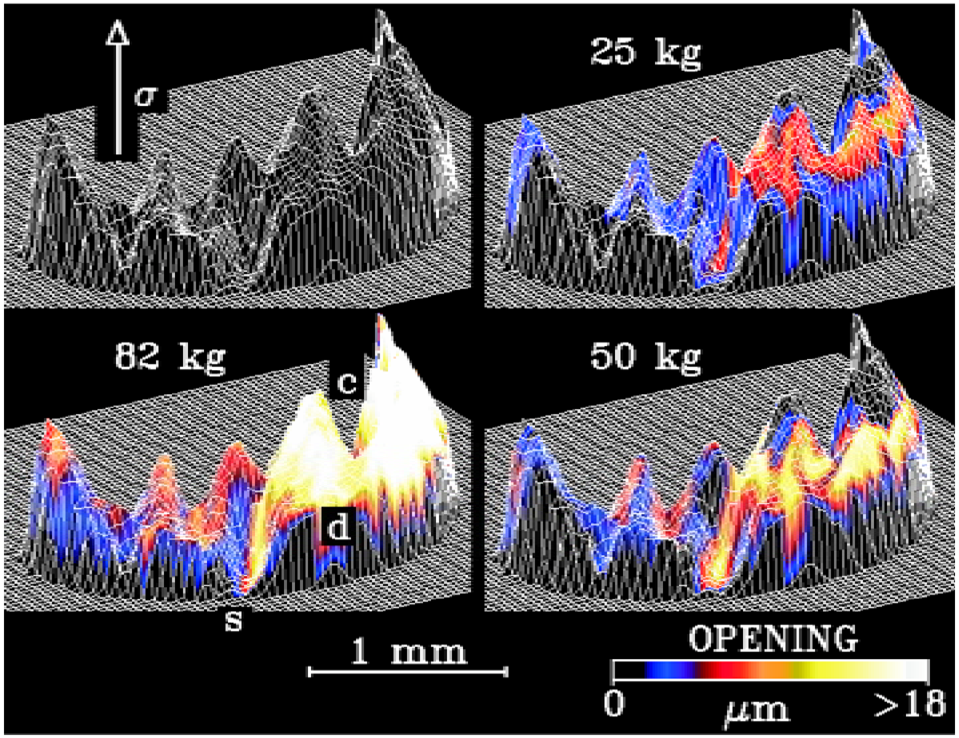
none particle roughness



Use x-ray microCT to measure crack opening as a function of 3-D position, applied load

Circumferentially notched cylindrical sample of Al

Mesh:
3D position
of crack

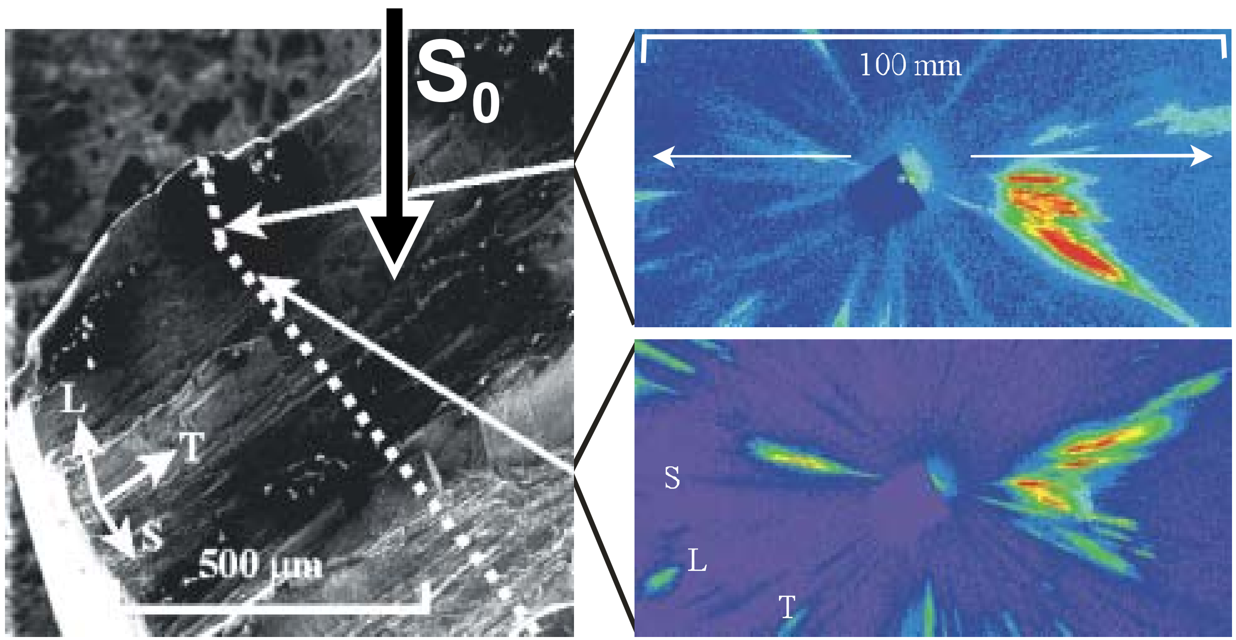


Color:
crack
opening

View: sample exterior from crack initiation point

Highly nonplanar crack path associated with closure. Why this path?

Groups of 5-10 adjacent pancake-shaped grains with nearly identical crystallographic orientations



crack surface

microbeam diff. patterns

Acta Mater 46 (1998) 4791-4799. ASTM STP 1359 (1999) 160-173.

The proteins in sea urchin teeth and in mammalian teeth (human, mouse, rat) react to the same antibodies. Therefore, relatively small differences in mineral-related protein control whether apatite (calcium phosphate, vertebrates) or calcite (calcium carbonate, echinoderms) is formed.

Easy to grow large calcite crystals (mm or greater). Hard to grow apatite larger than nanocrystals.

Hypothesis: Nanocrystal plus collagen composite gives greater flexibility in forming complex systems than does complex calcite crystal geometries.

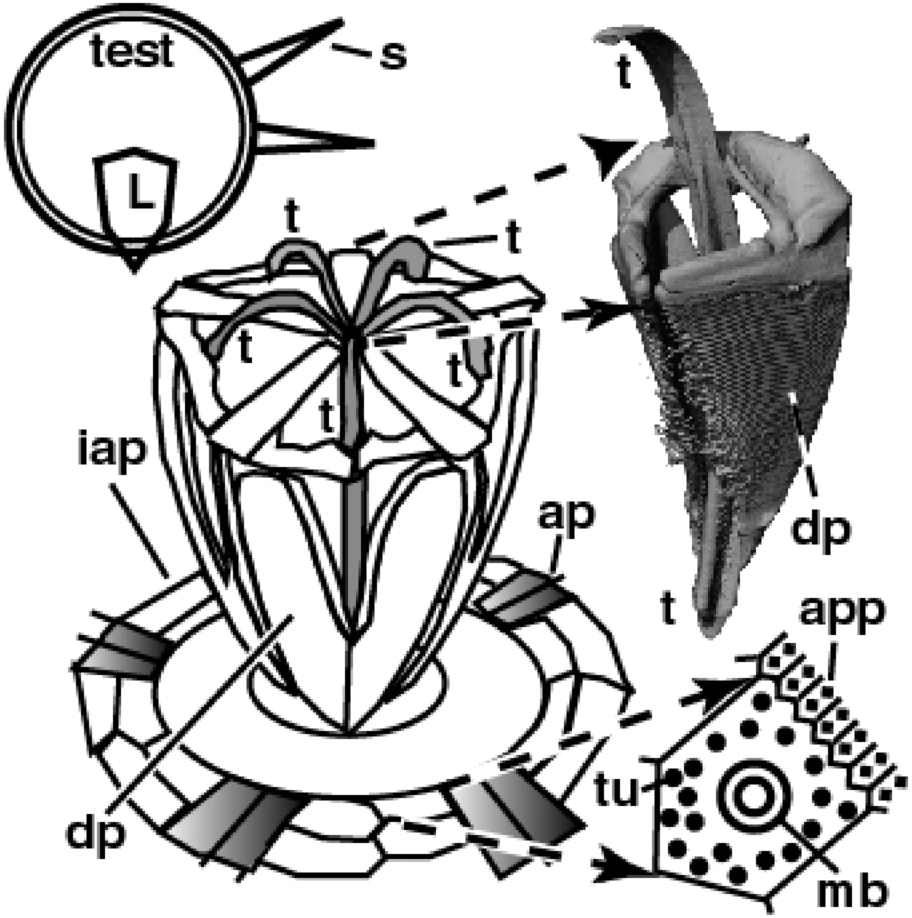
Advantage: Small changes in protein produce large changes in resulting product (bio-structural material).

Spatial distribution of protein ⟺ **Microarchitecture, Mineral density**

- Bone can be regarded as a discontinuously reinforced composite.
- Sea urchin ossicles can be regarded as 3D continuously reinforced composites with complex reinforcement geometry.

Aristotle's lantern, other ossicles

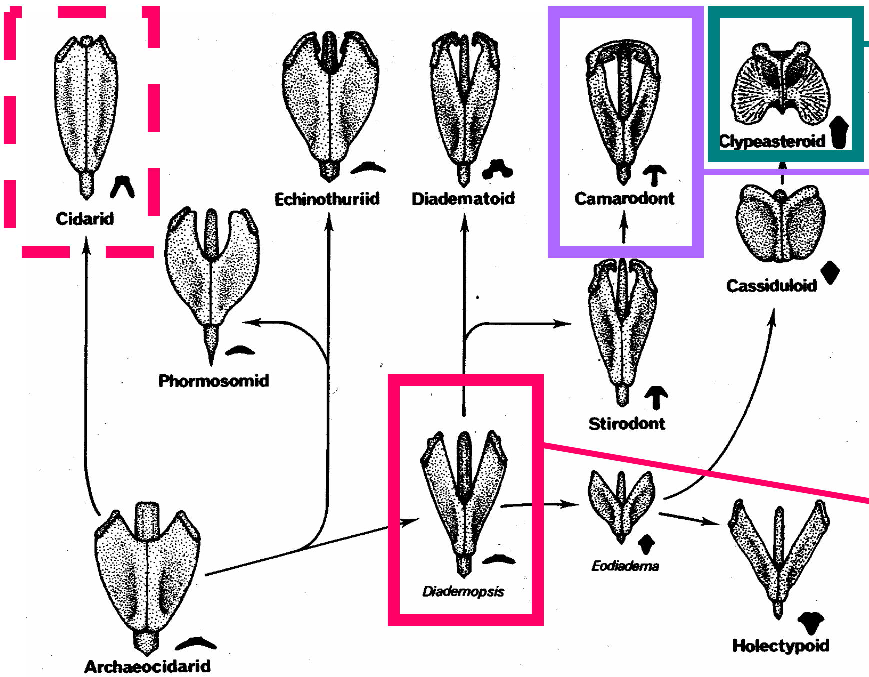
Lytechinus variegatus



dp : demi-pyramid
t : tooth
s : spine

Lantern design: scraping, crushing

- **Lighter lantern: less energy expended during feeding**
- **Stronger, stiffer lantern: more efficient feeding**



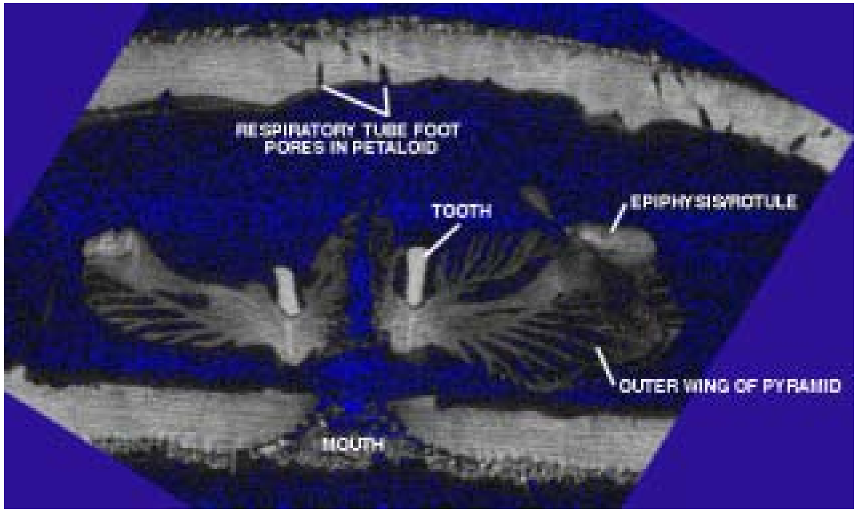
crushing lantern

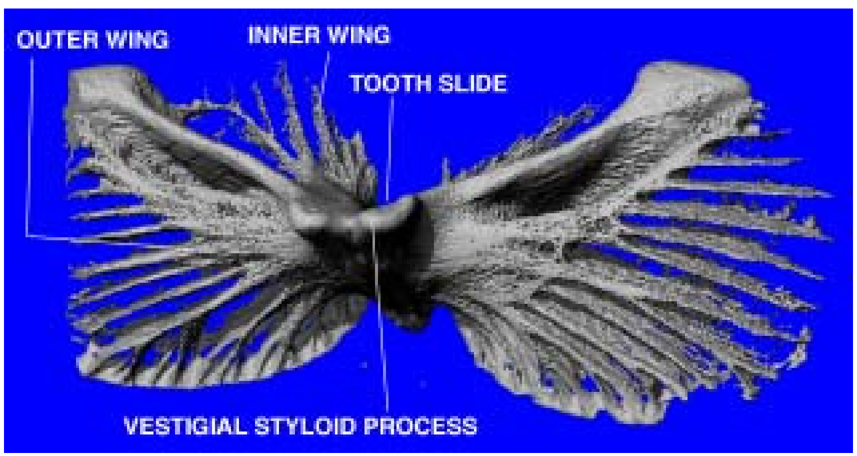
<u>Stiffen lantern</u> via: cross-brace top of foramen magnum

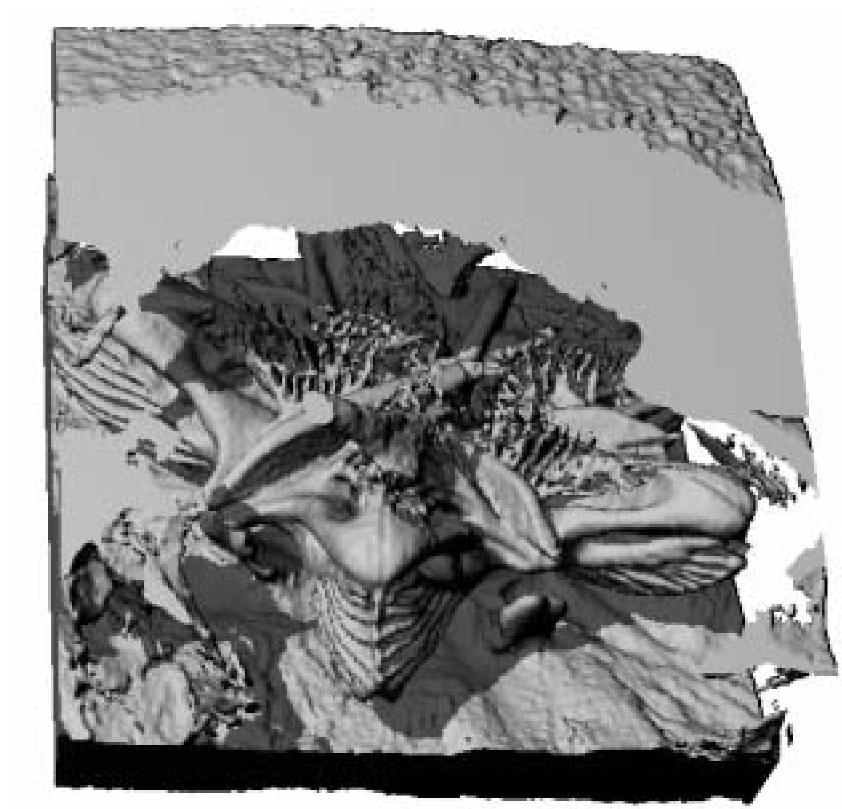
<u>Lighten lantern</u> via:
*** foramen magnum**
*** intrapyramidal cavity**

From Andrew Smith, *Echinoid Palaeobiology* (1984)

Example: Sand dollar pyramid







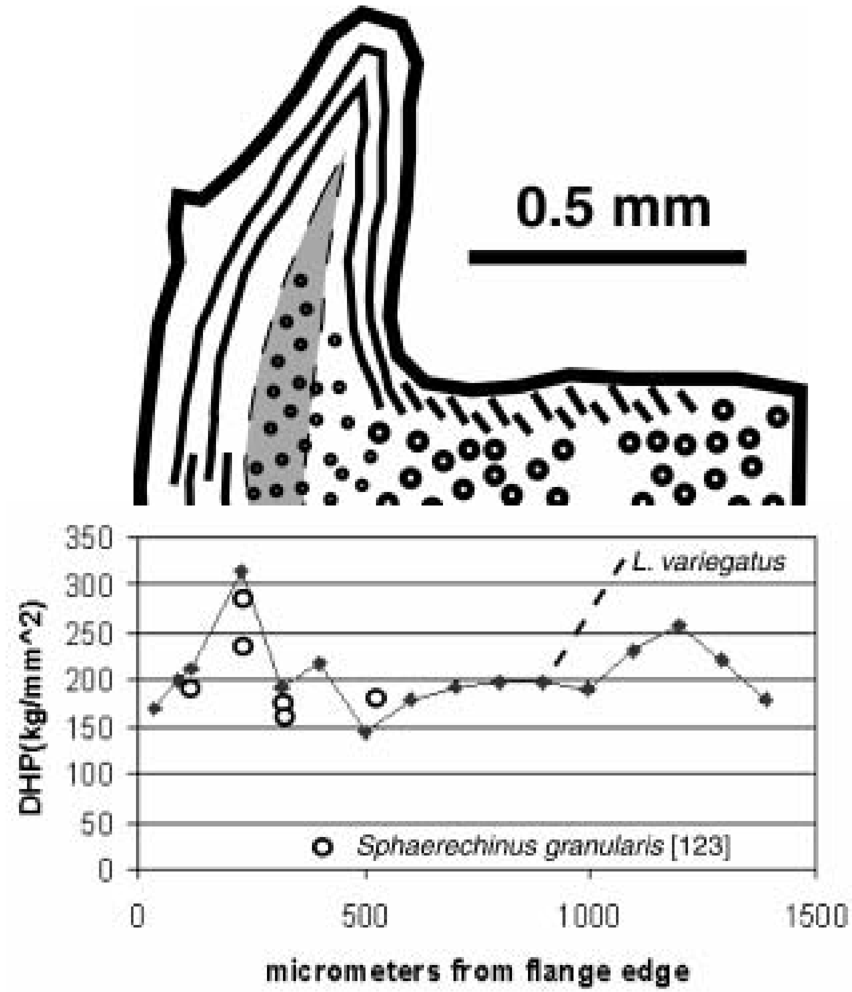
Sample from and labeling of images by R. Mooi, California Academy of Science.

To reinforce their ossicles (bone analogs), the sea urchins use all composite reinforcement tricks used in human-engineered composites.

- Solid solution hardening via $Ca_{1-x}Mg_xCO_3$ with varying *x.*
- Alignment (crystallographic) of reinforcing elements.
- Inclusion toughening of reinforcements.
- Interface tailoring.
- Functional gradients in reinforcement
  - Morphology
  - Composition
  - Dimensions
- Continuously growing teeth
  - Self-sharpening design
  - Gradual densification

Lytechinus variegatus

Vickers microhardness vs. position



Collaborators

- **A. Veis,** Cell and Molecular Biology, NU
- **K. Ignatiev,** GT
- ***Synchrotron microCT:*** **F. DeCarlo,** XOR-APS
- ***Diffraction mapping:*** **J. Almer,** XOR-APS

Grand Challenges

Grand Challenges

- Staying awake after dinner.

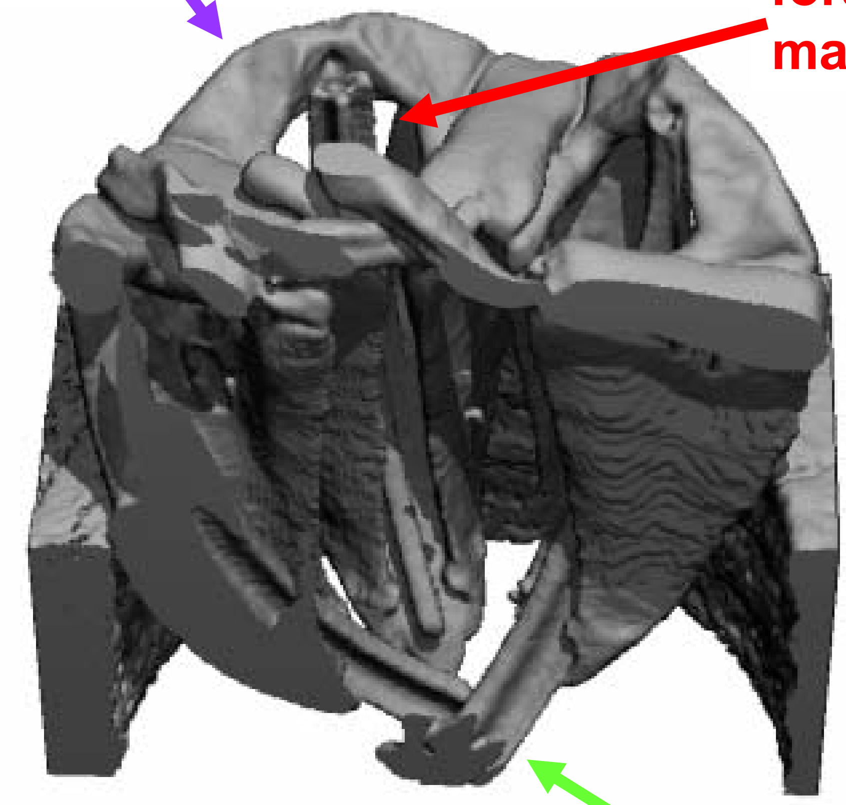
Grand Challenges

- Staying awake after dinner.
- Mapping the “design” space for a biocomposite system; transferring the design rules to engineering composites.
- Understanding the phylogenic relations between different orders/families/genera of sea urchins.
- Applying the same ideas to studying bone.

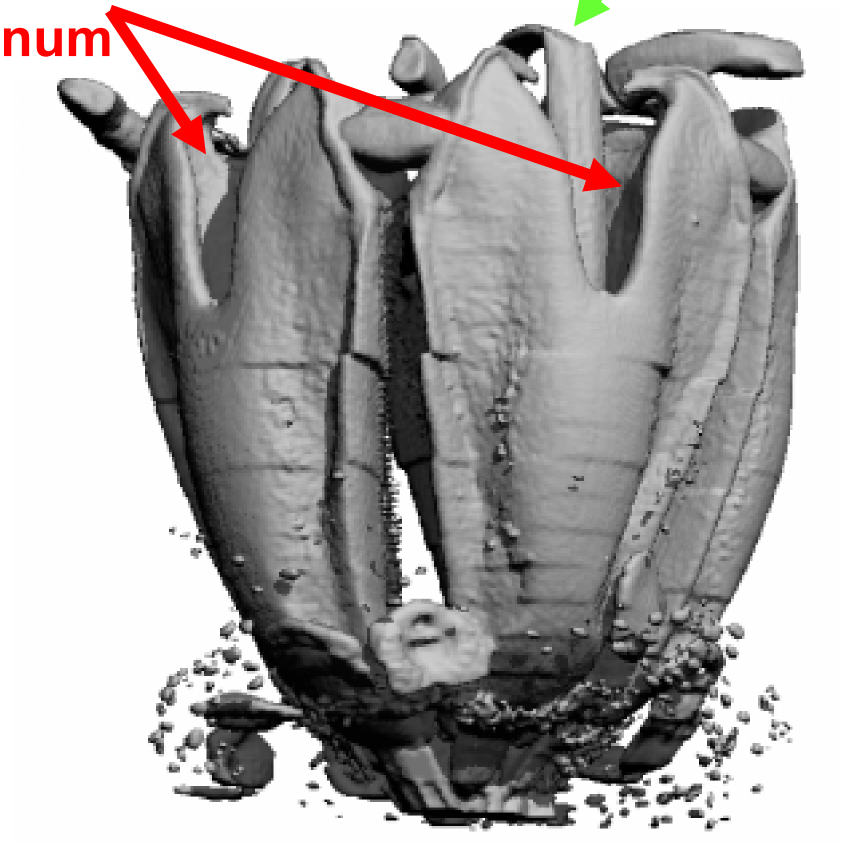
Areas in bone research where these ideas are applied.

- Regeneration of limbs in newts.
- Effect of resorption agents on bone.
- Aortic valve calcification.
- Bone formation in arthritis.
- Calcified nodule formation in juvenile dermatomyositis.
- …

Sea urchin lanterns



Paracentrotus lividus
(camarodont)



Arabacia punctulata
(stirodont)

Camarodont sea urchins

- **Data in the literature indicates urchins from different families have different reinforcement compositions *x*, dimensions, morphologies.**

- **Hypothesis: Different families of sea urchins produce functional teeth using different combinations of parameters.**

- **Studying teeth from different families is a probe of the "design space" available for calcite-based teeth.**
  - **Reveal interplay of reinforcement mechanisms**
  - **Guide design in engineering of structural tissue incorporating biomimetic strategies**

Sea Urchin orders with “T”-shaped teeth*

- **Salenoida**
- **Phymosomatoida** — *Stomopneustes variolaris*
- **Arbacacioida** — *Arbacia punctulata*
- **Temnopleuroida** — *Lytechinus variegatus*
- **Echinoida**** — *Paracentrotus lividus*, *Echinometra mathaei*, *Heterocentrotus trigonarius*, *Strongylocentrotus franciscanus*

*** After A.B. Smith (1978) Palaeontology 21(4) ?-789.**
**** Three families included.**

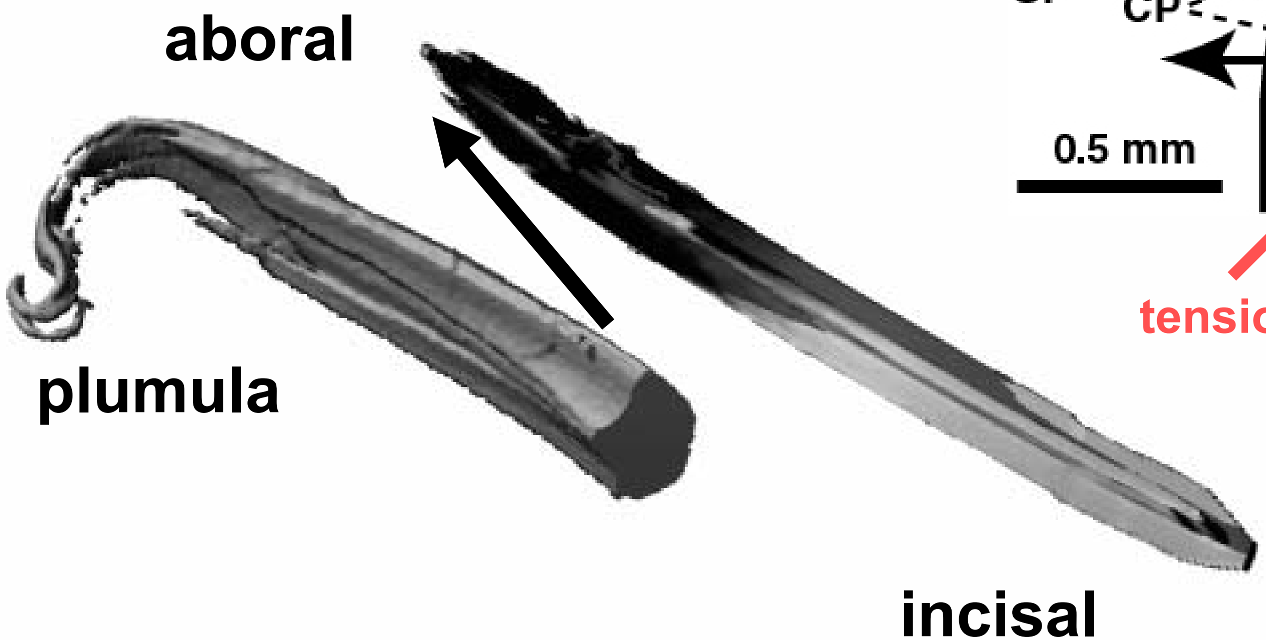
L. variegatus: "T"-shaped teeth for scraping action…analogous to "T"-shaped girder this shape resists bending

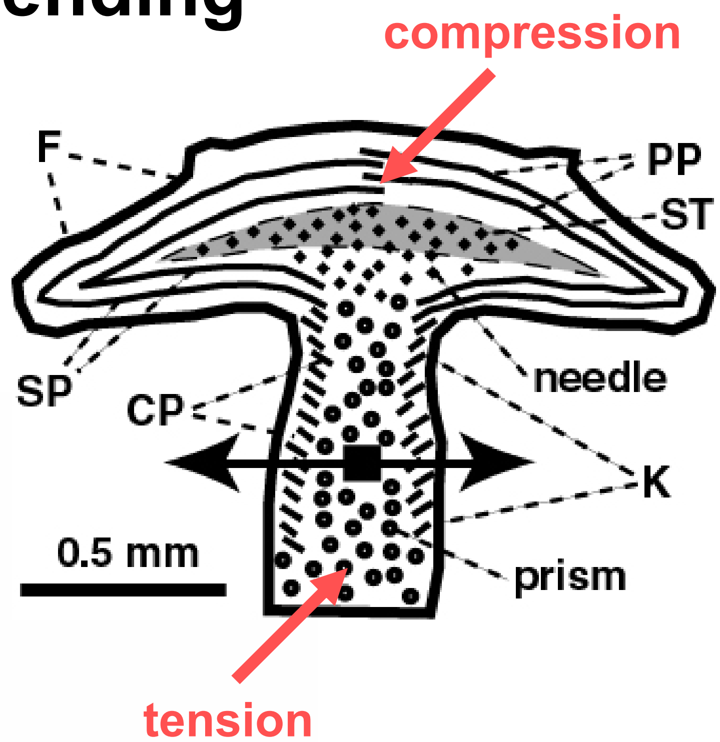
<u>Structural elements</u>

PP primary plates

SP secondary plates

CP carinar process plates





<u>Tooth parts</u>

F flange

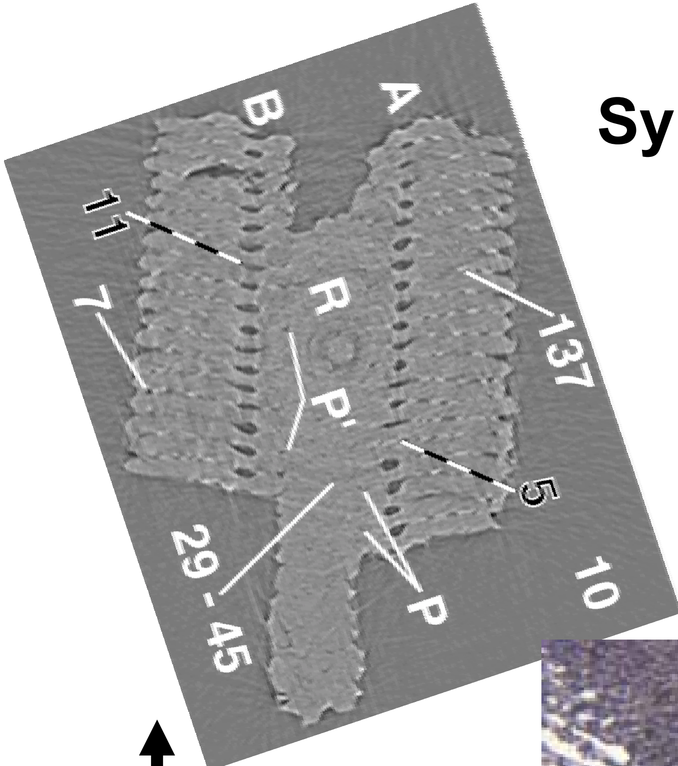
K keel

ST stone part

Camarodont sea urchins

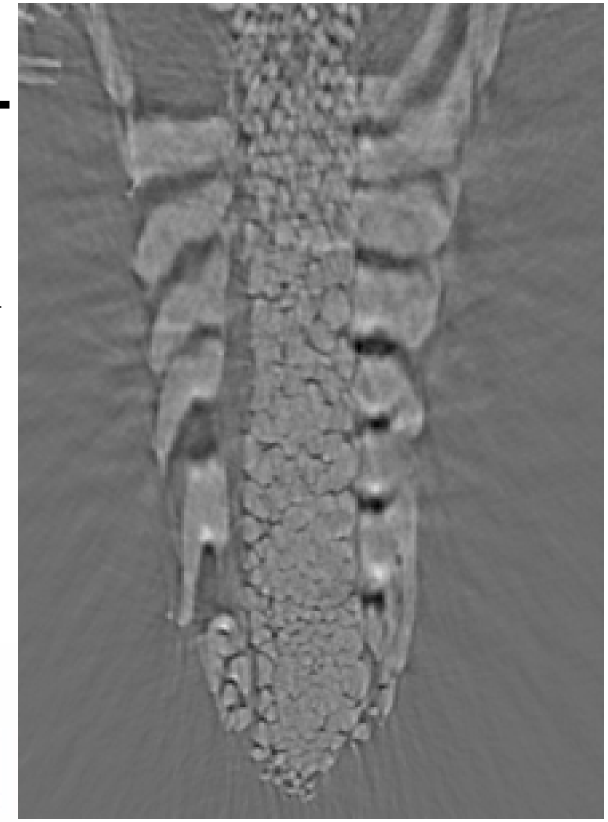
- Different families (genera?) vary composite design
- Data in the literature indicates that each family has optimized (?) functionality by giving different weight to the different sources of toughness, strength
- Characterizing teeth from different families may
  - Reveal interplay of reinforcement mechanisms
  - Guide design in engineering of structural tissue incorporating biomimetic strategies

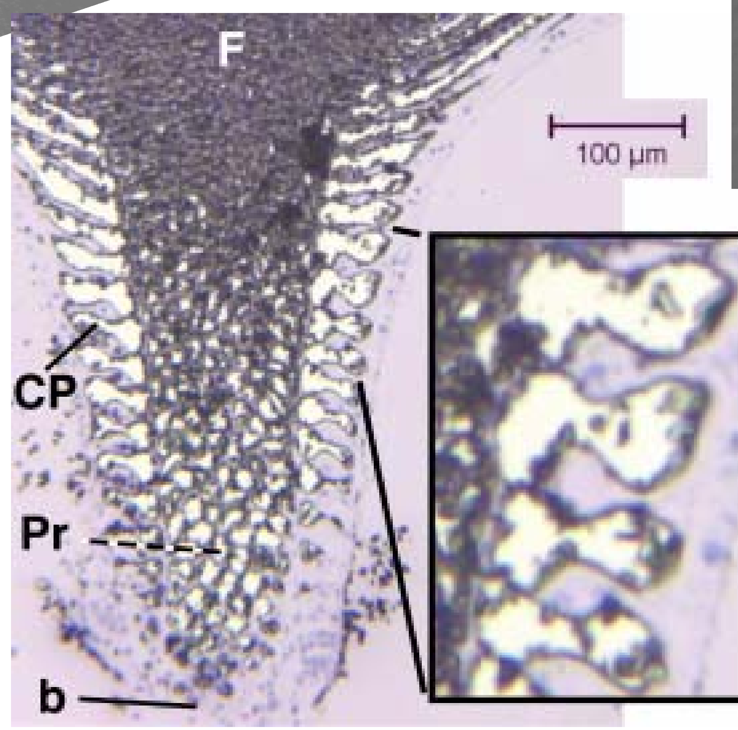
Synchrotron microCT



Keel fragment, 14 keV, 2.6 µm voxels, 512 x 512 reconstruction

Intact keel, 16 keV, 1.6 µm voxels, 1024 x 1024 reconstruction





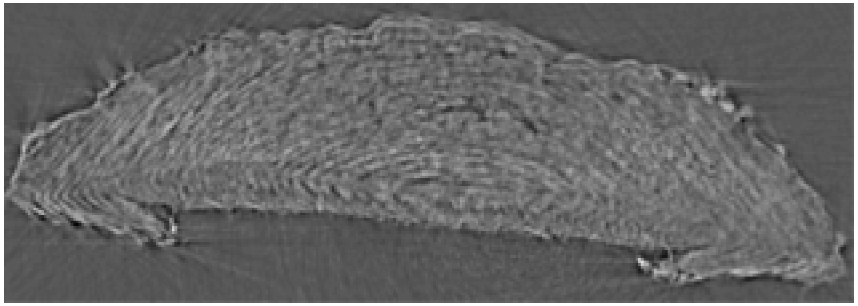
Optical thin section of keel and channels

Lytechinus variegatus tooth

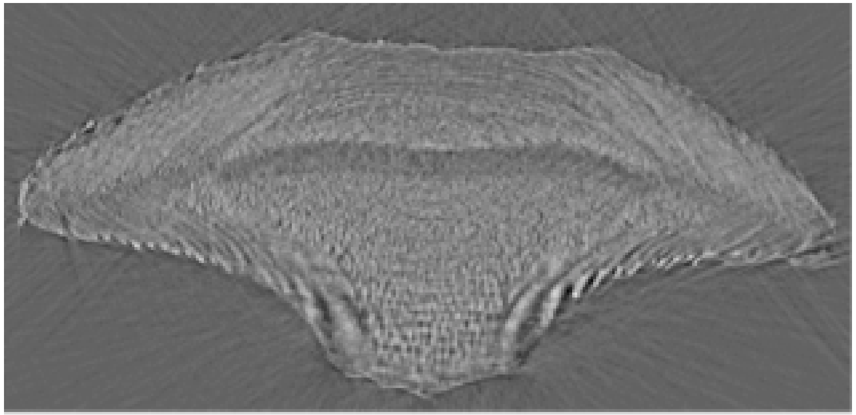
<u>Synchrotron microCT</u>

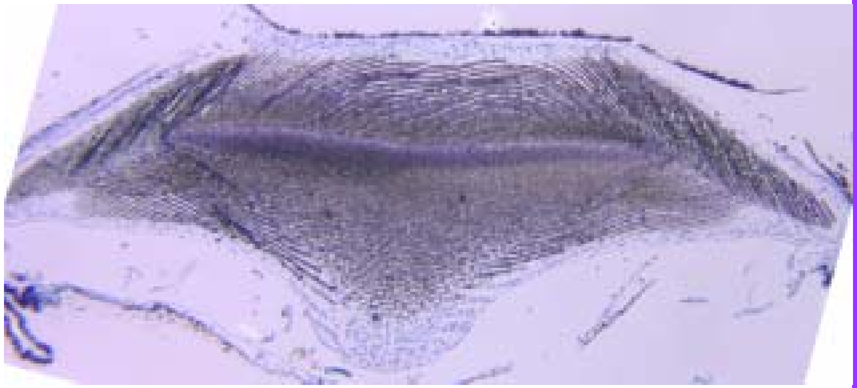
14 keV, 1.6 µm voxels, 1024 x 1024 reconstruction

mid plumula

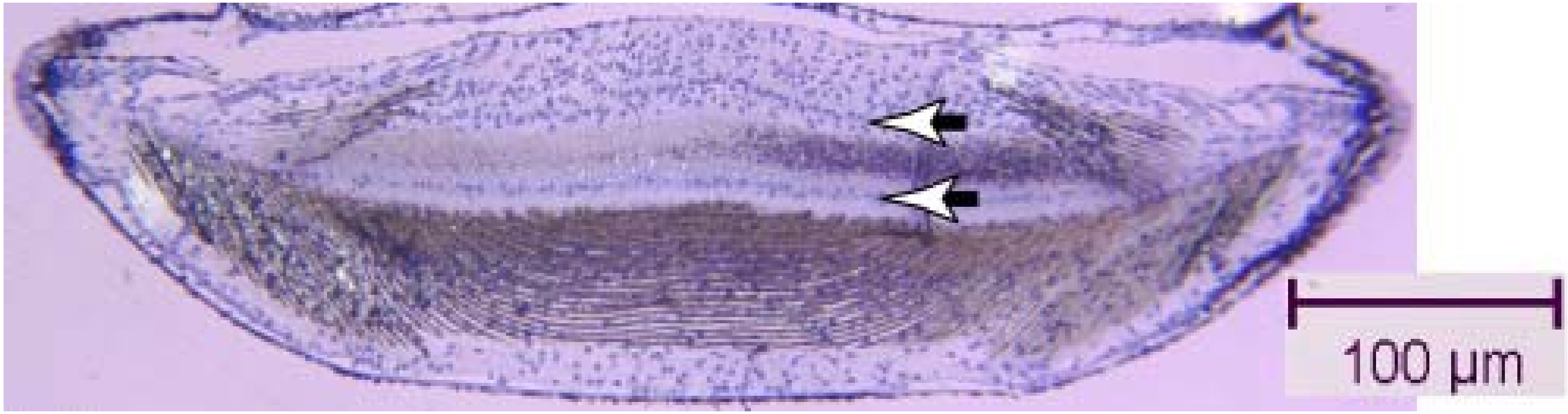


<u>Optical thin section</u>





plumula-shaft transition zone

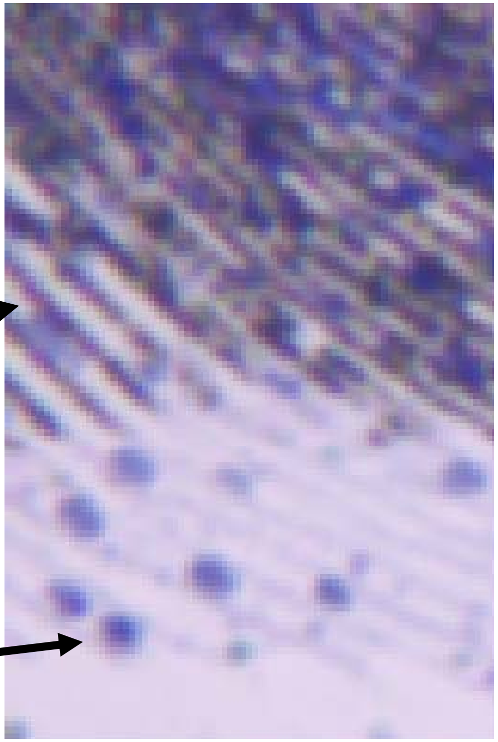


Thin section of tooth

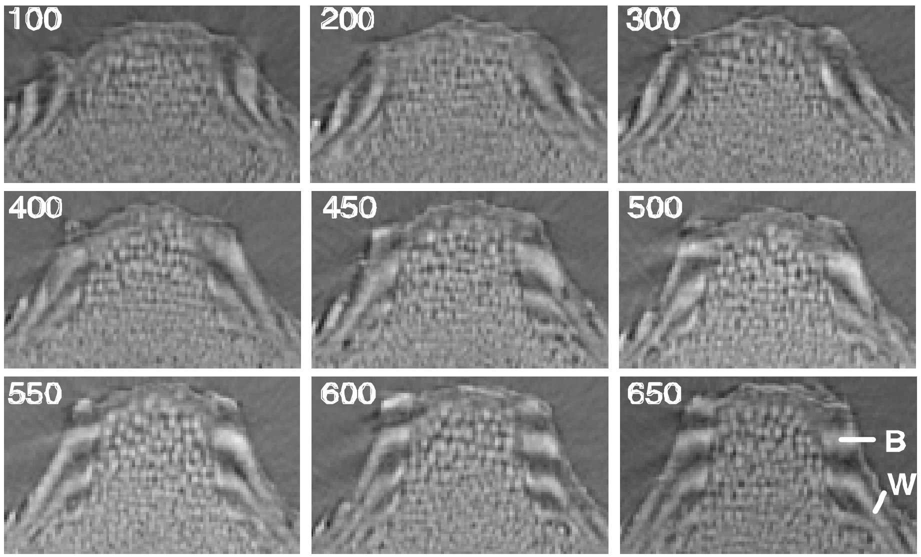
mineral (primary plates)

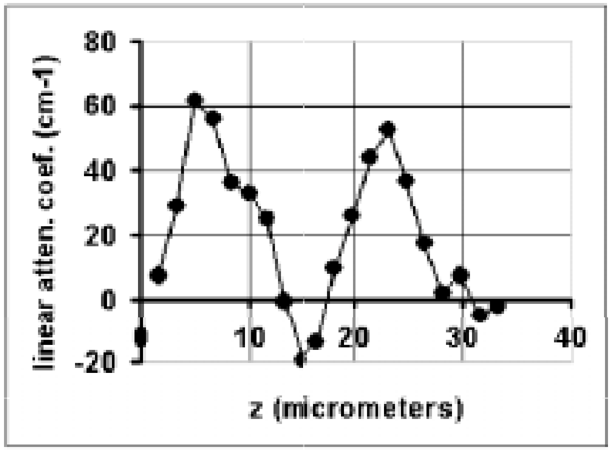
Syncitia

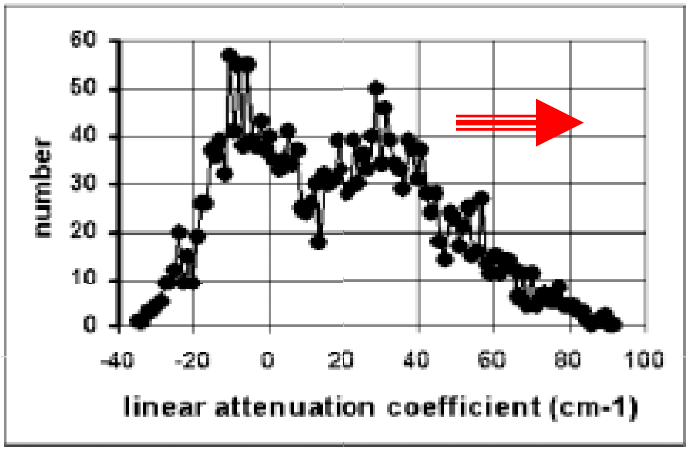
cell nuclei

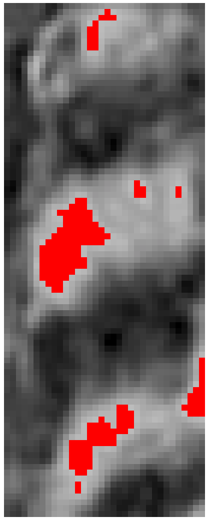


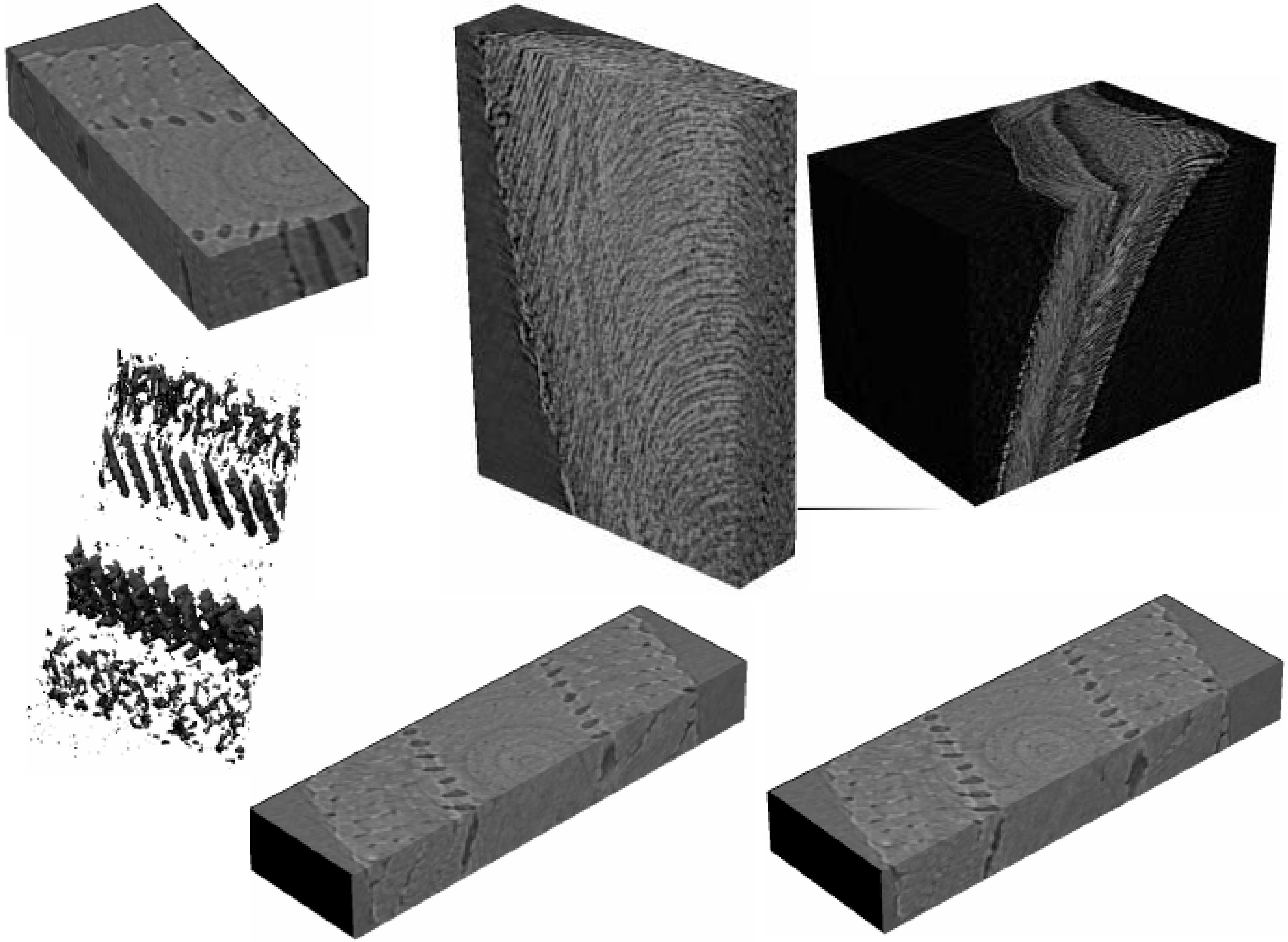
Values of linear atten. coeff. can be used but x-ray refraction effects are present!





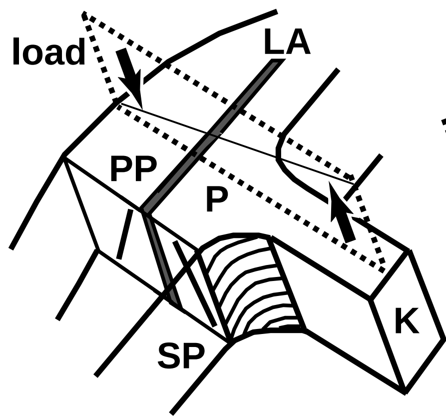


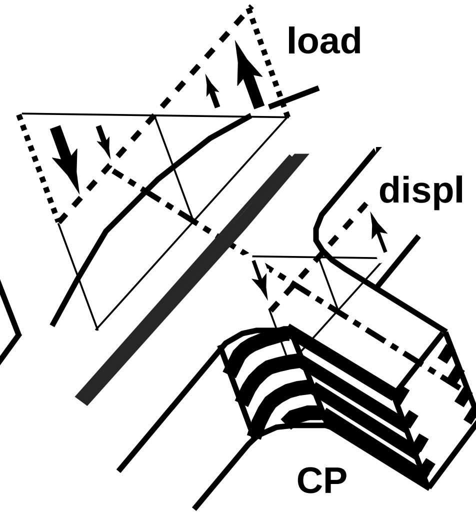




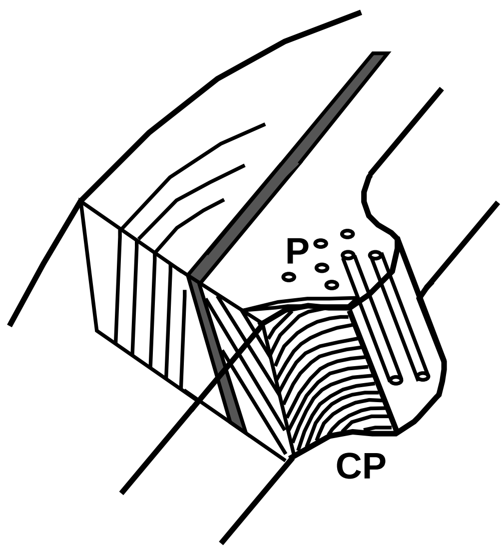
Lytechnius variegatus: Possible function of carinar process plates nearly perpendicular to tooth axis.

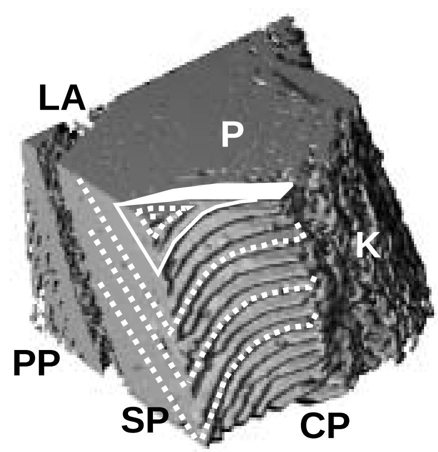
primary bending



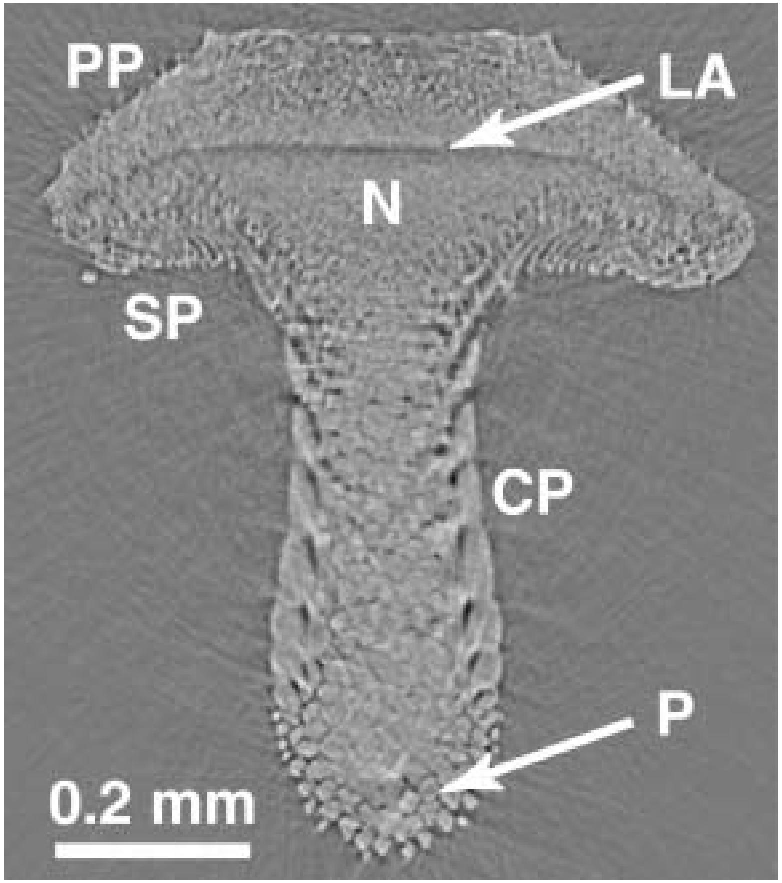


secondary, i.e. lateral, bending… grazing on a laterally tilted surface

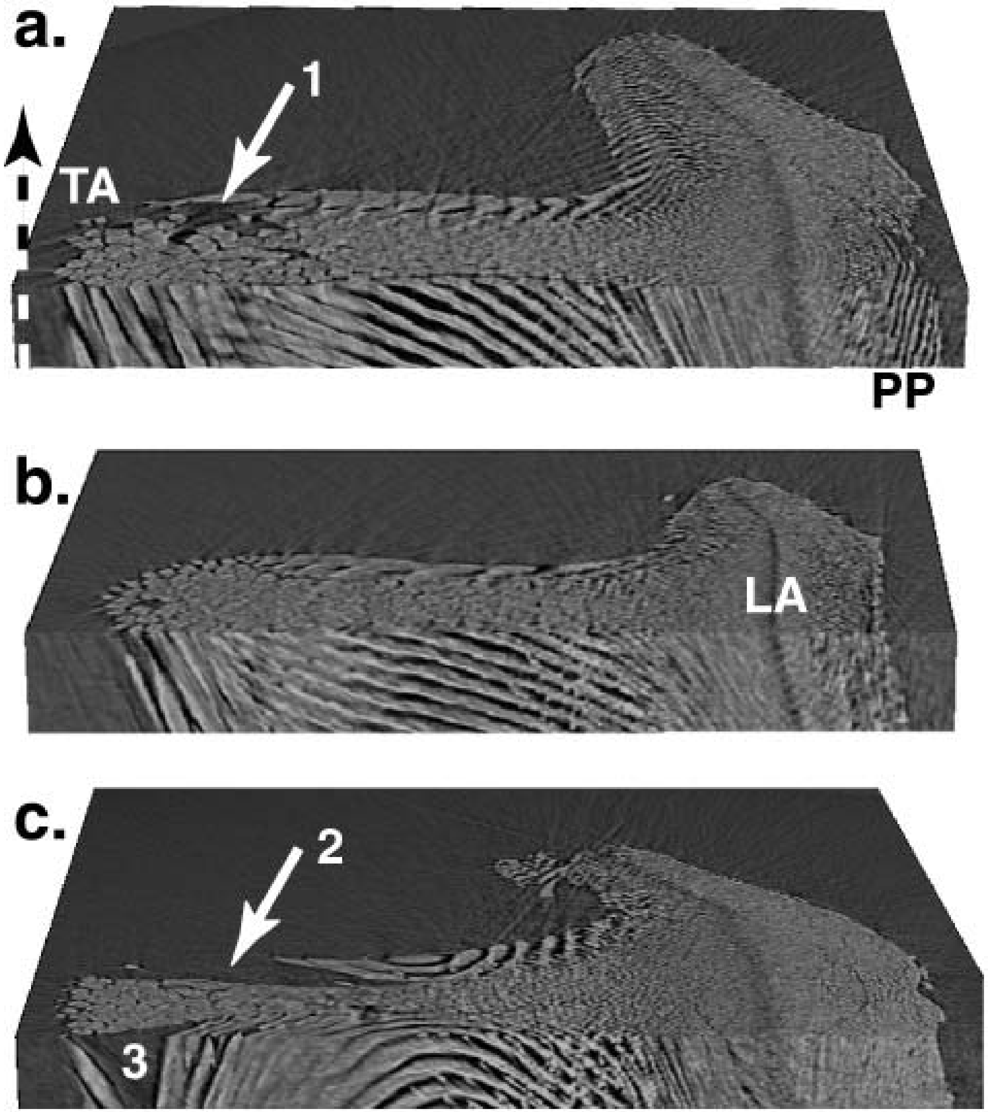




In different phylogenic orders, families of camarodonts: Are carinar process plates aligned to resist transverse bending?



- Slice of *Heterocentrotus trigonarius*, 1.66 µm voxels, 21.2 keV (left).
- Compare orientations of carinar process plates for three families of one order (Echinoida):
  - Strongylocentrotus
  - Echinometridae
  - Echinidae

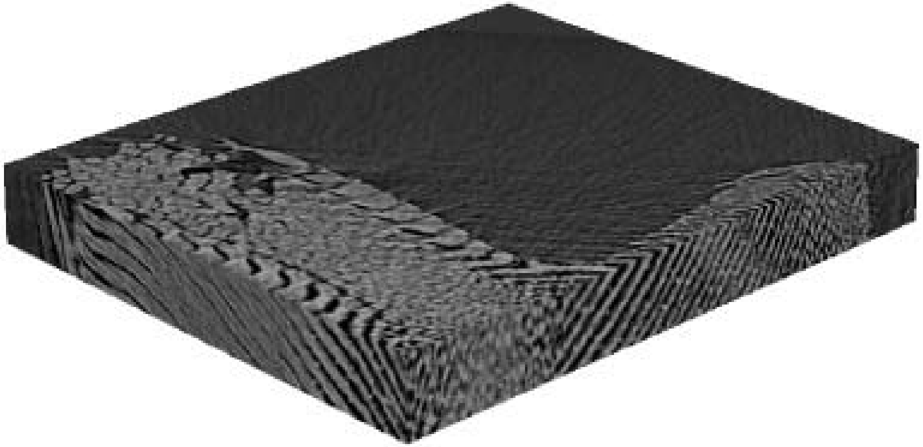


Strongylocentrotus franciscanus
(Strongylocentrotus)

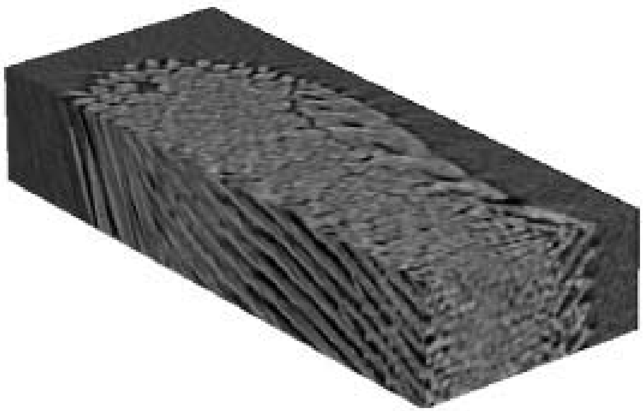
Heterocentrotus trigonarius
(Echinometridae)

Paracentrotus lividus
(Echinidae)

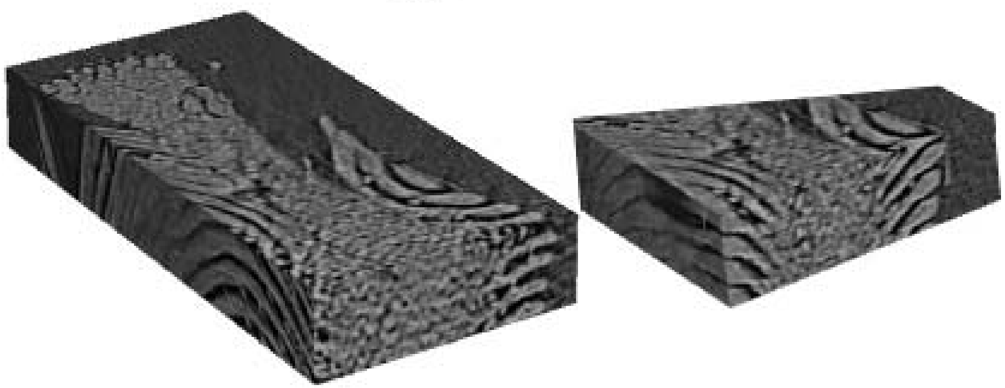
1.66 µm voxels, 21.2 keV, stack of 50 slices along TA.



Strongylocentrotus franciscanus
(Strongylocentrotus)

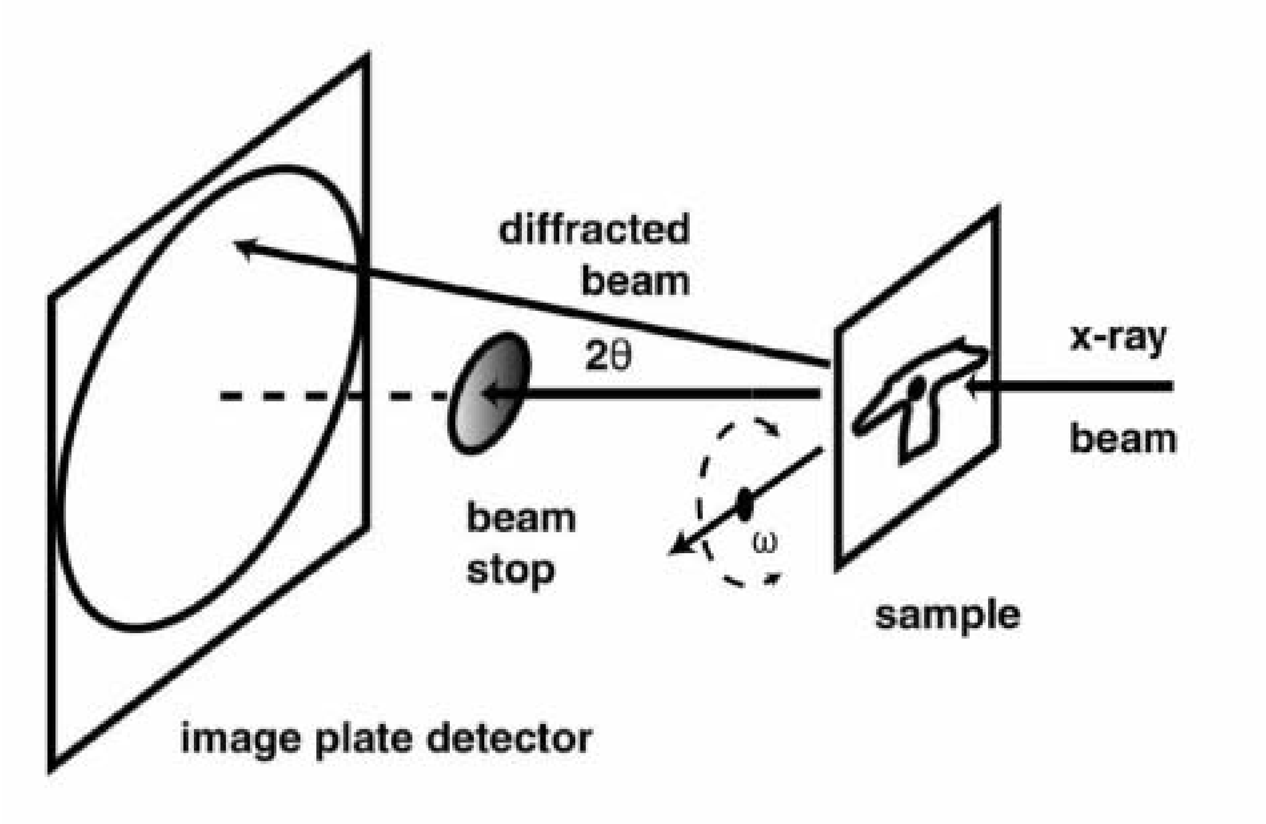


Heterocentrotus trigonarius
(Echinometridae)

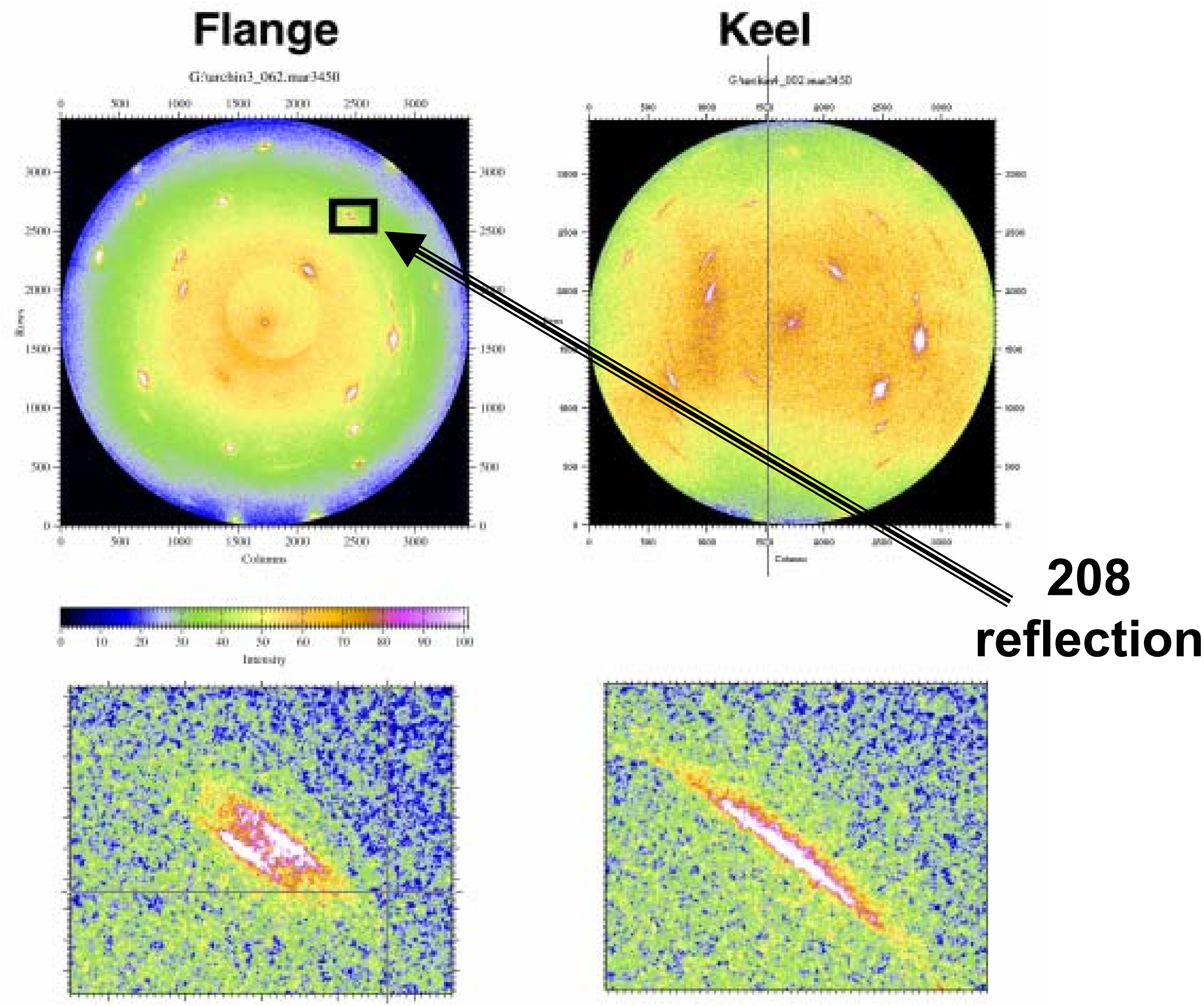


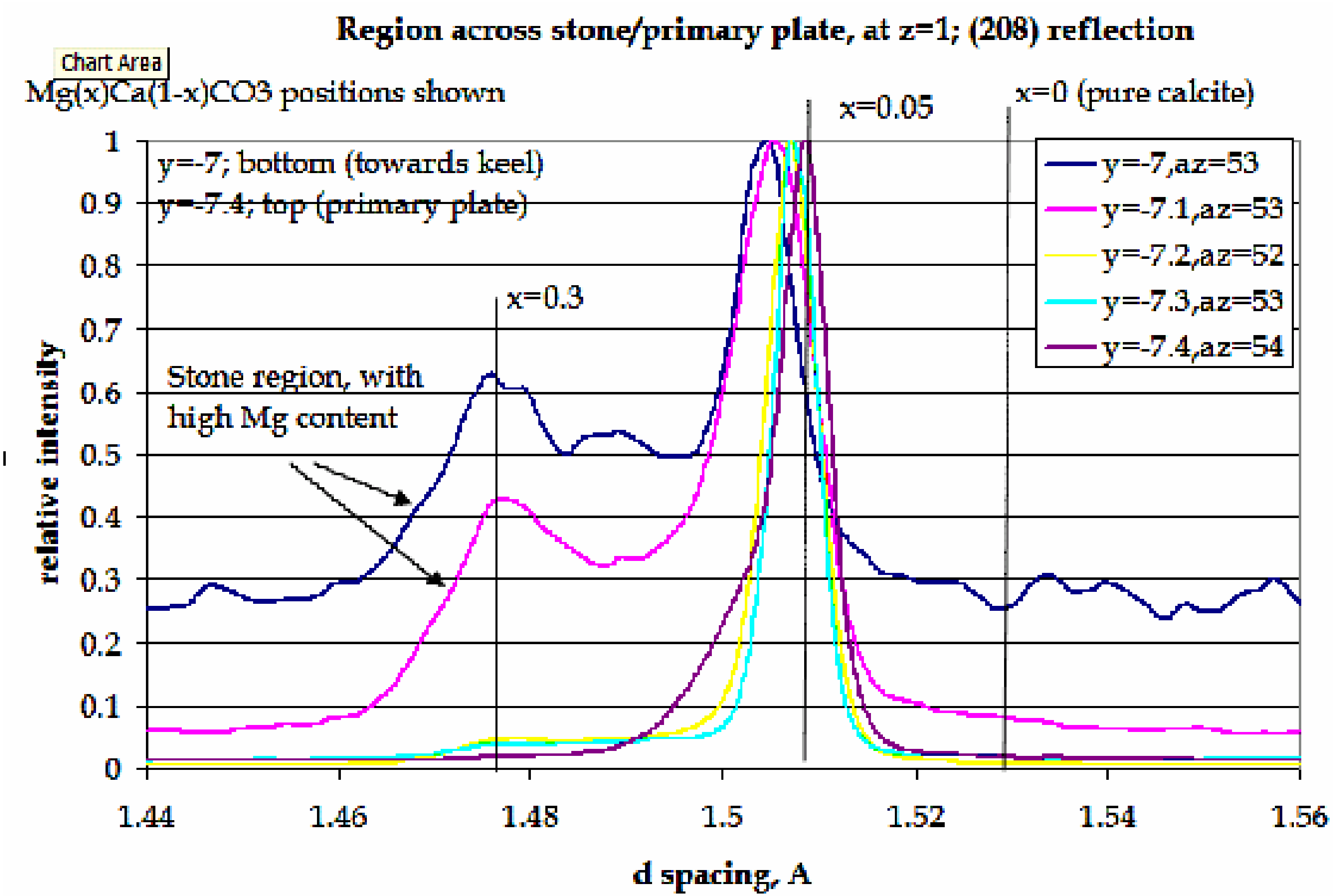
Paracentrotus lividus
(Echinidae)

Microbeam diffraction mapping



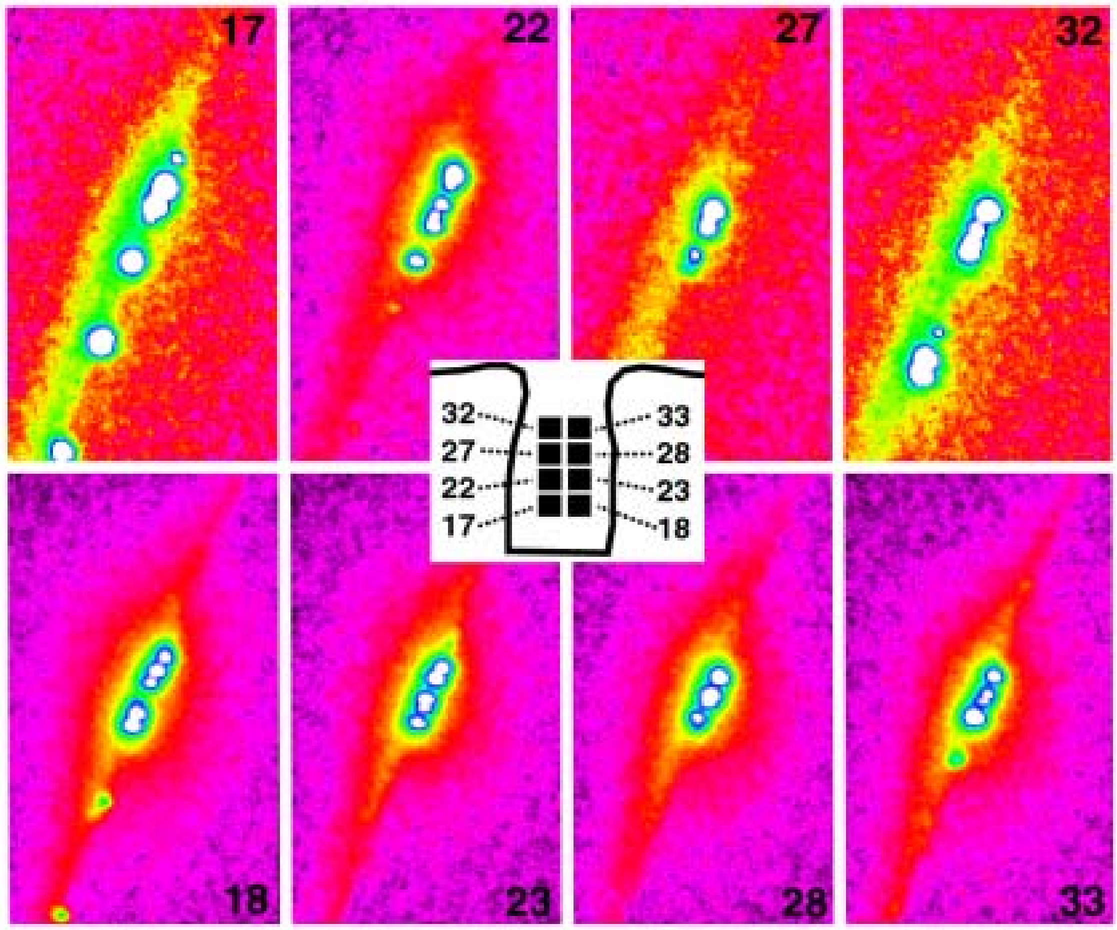
APS, 1-ID, 80.8 keV,
beam size down to 20 μm
MAR IP detector


Flange
Keel
G:\urchin3_062.mar3450
Rows
Columns
Intensity
208
reflection

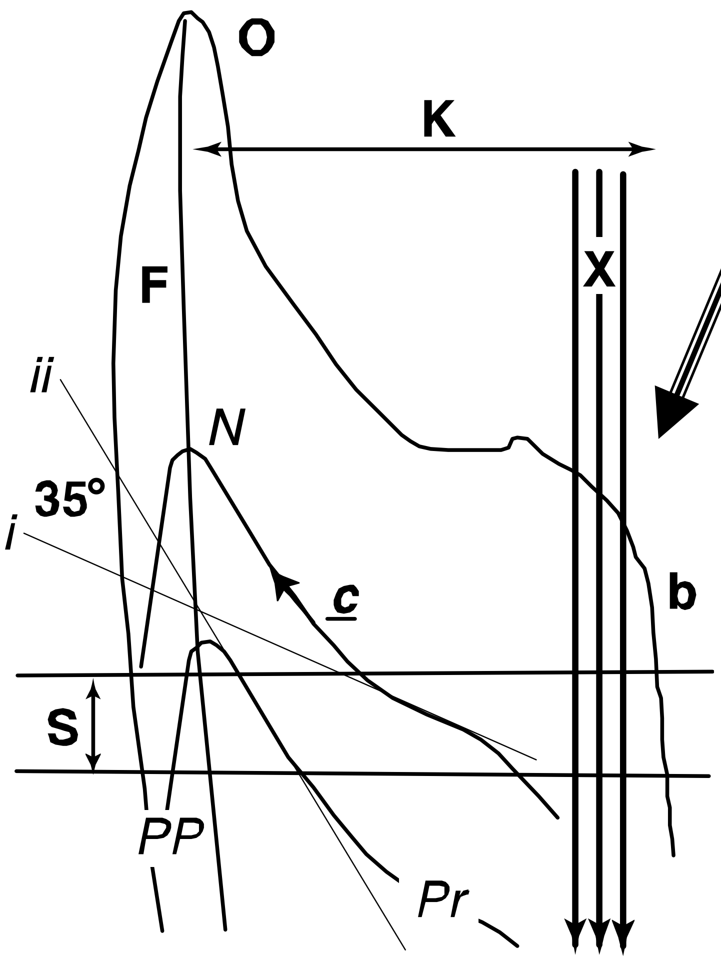


Region across stone/primary plate, at z=1; (208) reflection
Chart Area
Mg(x)Ca(1-x)CO3 positions shown
x=0.05
x=0 (pure calcite)
y=-7; bottom (towards keel)
y=-7.4; top (primary plate)
x=0.3
Stone region, with
high Mg content
y=-7,az=53
y=-7.1,az=53
y=-7.2,az=52
y=-7.3,az=53
y=-7.4,az=54
relative intensity
d spacing, A
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1.44
1.46
1.48
1.5
1.52
1.54
1.56

Discrete diffraction spots as a function of position in the keel



Range of calcite orientations in *Paracentrotus lividus*



Fiber orientations vary ~ 35 ° in keel, Wang *et al.* (1997) Phil Trans Roy Soc B352, 469-480.

Fiber orientations rotate ~ 15° in *Lytechinus variegatus* keels (from diffraction data).

Same rotations in *P. lividus*, *S. franciscanus*, *H. trigonarius.*

Diffraction mapping results for *L. variegatus*

- Two populations of crystals with different Mg contents (13 and 32 mol. %, respectively) in flange, appear to be epitaxially related.
- One population of crystallites in keel, same composition as low Mg crystallites in flange.
- Individual Mg prisms mapped in keel.
- Crystallite size, microstrain broadening observed.
- Between keel and flange crystals rotate ~15°.
- Note 80.8 keV photons suffer ~60% attenuation passing through 2 cm calcite.

Diffraction + microCT

- **Low attenuation zone in flange is a common feature in “T”-shaped teeth from different orders.**
- **Different flange designs and different prism configurations seen (*Lytechinus variegatus vs. Paracentrotus lividus*).**
- **Low attenuation zone in flange consists of ~ 25-30 vol. % non-mineral (*Lytechinus variegatus*) from consideration of the composition (diffraction) and experimental linear attenuation coefficients.**
- **Low attenuation zone gradually fills in as the incisal end is approached.**

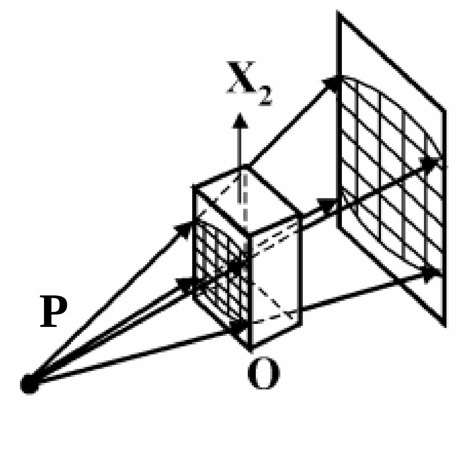
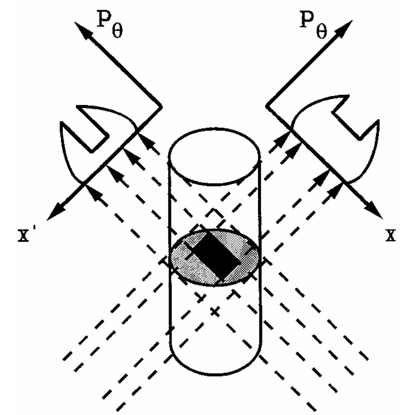
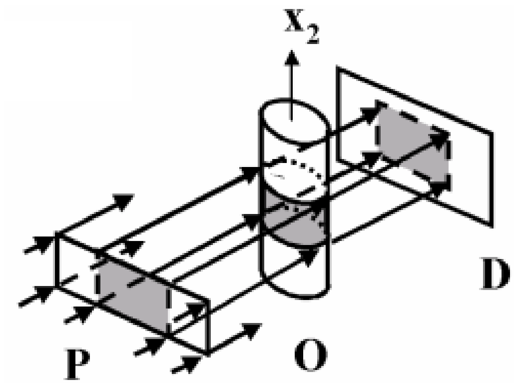
Conclusions

- **Spatial resolution of synchrotron microCT required to resolve individual plates in sea urchin teeth.**
- **Considerable variation in carinar process plate morphology in Order Echinoida.**
- **In all three families, plates appear to be oriented to resist secondary bending.**
- **Require submicron resolution (perhaps 10 nm) to image inclusions (macromolecules) within plates, prisms.**
- **Require submicron resolution to image “cement” between plates and prisms.**
- **Require 2 μm over FOV of 2 cm to do *in vivo* imaging of sea urchin lantern.**

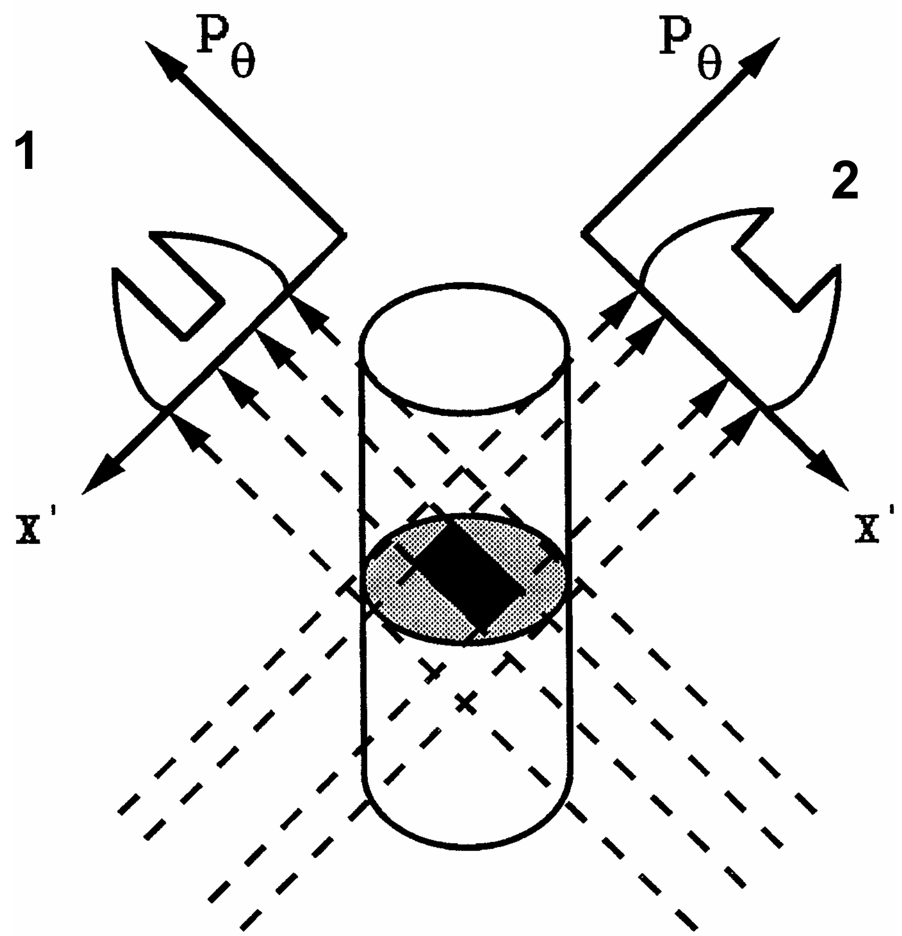
- Require ~ 10 nm resolution for discrimination of mineral crystallites in bone.
- Simultaneous imaging with diffraction would be a real plus.
- Improved availability of phase imaging…resolve cartilage vs. muscle.
- Improved analysis tools for less experienced users.


Larson
Lake Geneva
OK, stranger... What's the circumference of the Earth?.. Who wrote "The Odyssey" and "The Iliad?"..What's the average rainfall of the Amazon Basin?
Bart, you fool! You can't shoot first and ask questions later!

MicroCT

laboratory		synchrotron
		
10 µm, 10 mm	**<u>voxel size, FOV</u>**	**~1 µm, ~1 mm**
polychromatic	**<u>X-rays</u>**	**monochromatic**
40	**<u>slices / data set</u>**	**1000**
~ 15 min	**<u>time / data set</u>**	**~ 15 min**
(Scanco MicroCT- 40)		**(SRI-CAT, 2BM of APS)**

How reconstruction works (cartoon)

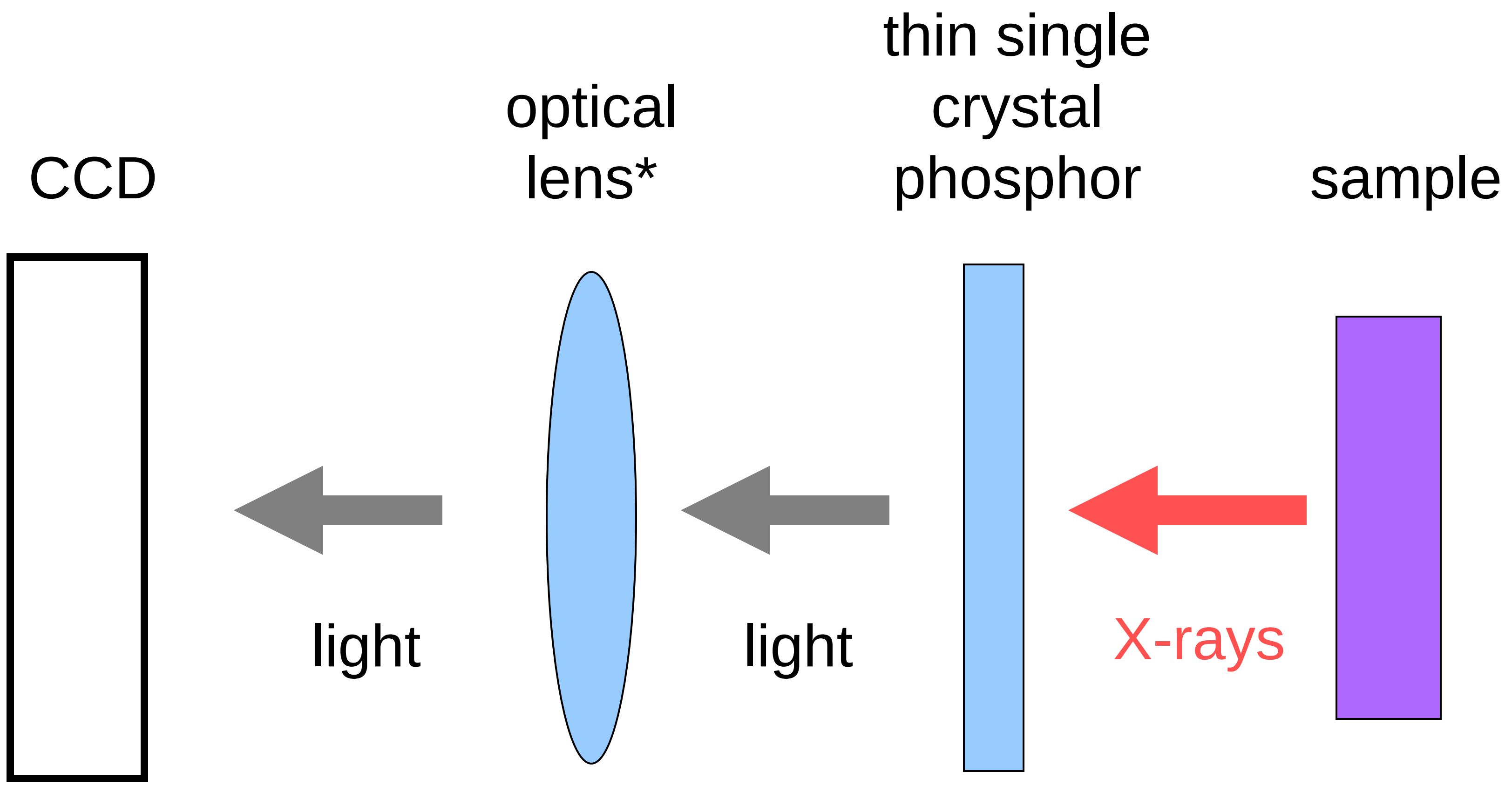


Along direction 1, the high absorption rectangle makes a spatially narrow but deep "valley" in the absorption profile P_θ.

Along direction 2, the profile P_θ has a spatially wide, but shallow "valley".

At intermediate angles the edges of the valley are less sharp.

Typical x-ray imaging set-up with synchrotron x-radiation



* Low depth of field, reject scattered light photons.

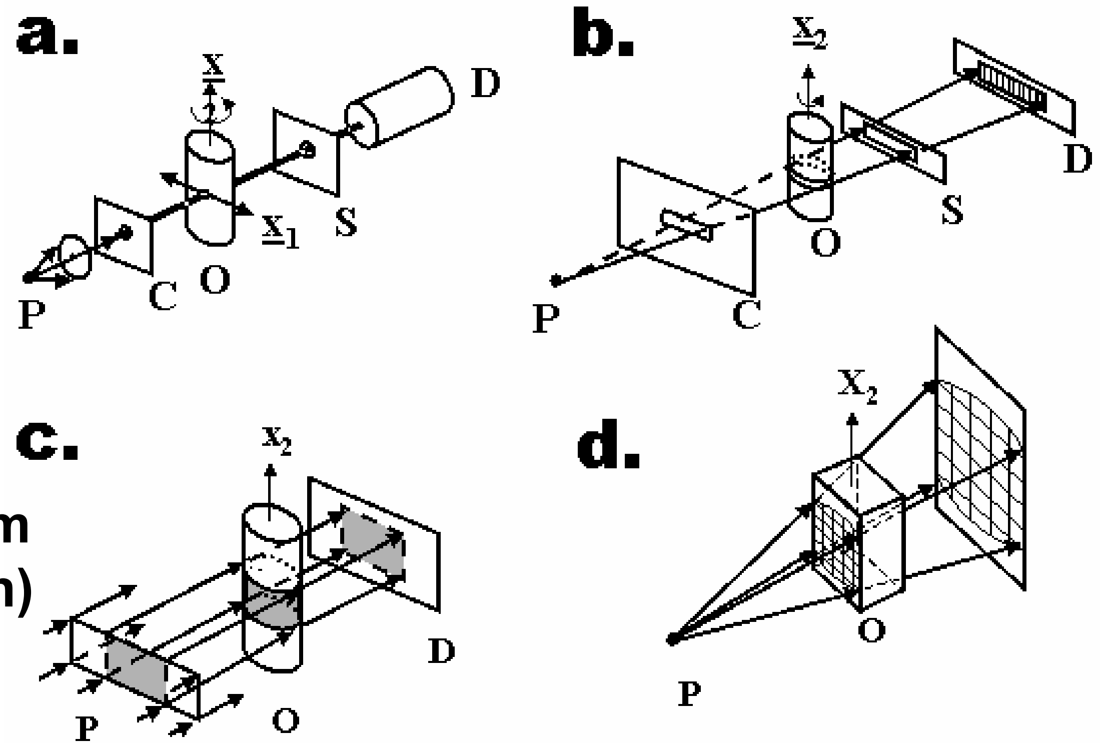
Experimental microCT approaches

Pencil beam

Fan beam

Parallel beam
(synchrotron)

Cone beam
(Scanco
MicroCT-40)



a. Very slow, no effect of scatter.
b. Somewhat faster than a., scattering affects fidelity.
c., d. Fastest, scattering affects fidelity.